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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Date of mailing (day/month/year) 03 December 1997 (03.12.97)	
International application No. PCT/US97/06891	**Applicant's or agent's file reference** 03063-0231WP
International filing date (day/month/year) 18 April 1997 (18.04.97)	**Priority date** (day/month/year) 19 April 1996 (19.04.96)
Applicant FIELDS, Howard, A. et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   18 November 1997 (18.11.97)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer B. Fitzgerald
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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
03063-0231WP		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US97/06891	18/04/1997	19/04/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/11		
Applicant		
THE GOVERNMENT OF THE U.S.A. AS ... et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/11/1997	13.07.98
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US97/06891

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-12,14-21, 24-108	as originally filed				
13,22,23	as received on	08/06/1998	with letter of	08/06/1998	

Claims, No.:

1-68	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-6,47-53,60,61,64-68.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3,5,6,47-53,60,61,64-68
	No:	Claims	1,2,4
Inventive step (IS)	Yes:	Claims	
	No:	Claims	3,5,6,47-53,60,61,64-68
Industrial applicability (IA)	Yes:	Claims	1-6,47-53,60,61,64-68
	No:	Claims	

2. Citations and explanations

   see separate sheet

Ad item IV:

Immunogenic peptides which are derived from different regions of various proteins of the HAV are disclosed in the art (see e.g. D1 to D3; EP-A-0421635; EP-A-0199480, WO 91/11460).
Therefore further peptides which are derived from different proteins of the HAV but also peptides which are derived from different regions of the same protein are no longer connected by a common inventive link.
Such a link could only be established for those peptides which share a common structural and **special feature** i.e. a sequence element by which the claimed peptides distinguish from the prior art. Such an element is neither recognisable for two (selected) peptides shown in SEQ. ID. 1 -72, let alone for a (selected) group or even for all of them.
Thus, each of the peptides claimed prima facies constitutes a single (alleged) invention.
Since no additional examination fees have been paid and no special feature with regard to a group of peptides has been indicated by the Applicant, this opinion is carried out on the claims insofar as they relate to SEQ ID NO. 4.

Ad item V:

The quoted document is:

(1) EP-A-0421635

D1 describes immunogenic peptides which comprise a sequence "which is substantially similar" to the sequence as disclosed in SEQ. ID No. 4 (see page 12, lines 1 to 10 of D1) and the corresponding DNA sequences.
In fact, the sequence of D1 differs merely by two amino acids from the sequence of SEQ ID NO. 4, namely those at position 14 and 20.
Thus the sequence of D1 is covered both by the wording of Claim 1("substantially similar") and Claim 2 ("binds to an antibody specifically (?) immunoreactive").
Therefore, at least Claims 1, 2 and 4 are not novel.

Claims 5 and 6, are novel over D1 in a strict sense.

However, the additional features within said claims must be considered as being plainly obvious modifications of the basic subject-matter claimed, and thus are devoid of any inventive activity.

Finally, Claims 3, 47-53, 60, 64 to 68 relate to the specific peptide shown in SEQ ID NO: 4 (and DNA sequences encoding said peptide or he use of said peptide) and, consequently, are also novel.
However, as outlined above even said specific sequence has only minor differences in comparison with the sequence of D1. Unless said differences do not result in an unexpected or advantageous effect over the prior art peptides, an inventive activity has to be denied. Such an effect,at present, is not recognisable. Therefore, none of the claims fulfils the requirements of Articles 33.2 and/or 33.3 PCT.

For the assessment of the present claims 50-51 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

09/171282

CORRECTED VERSION*

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ANTIGENICALLY REACTIVE REGIONS OF THE HEPATITIS A VIRUS POLYPROTEIN

(57) Abstract

The present provides immunogenic HAV peptides, antibodies and assays for detecting HAV. Vaccines against HAV are also provided.

*(Referred to in PCT Gazette No. 52/1999, Section II)

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length nucleic acid sequence as well as non-full length sequences derived from the full length sequence. It will be understood by those of skill that the sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell.

"Conservatively modified variations" of a particular nucleic acid sequence refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given peptide. Such nucleic acid variations are Asilent variations,@ which are one species of Aconservatively modified variations.@ One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each Asilent variation@ of a nucleic acid which encodes a peptide is implicit in any described amino acid sequence. Furthermore, one of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are Aconservatively modified variations@ where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

See also, Creighton (1984) *Proteins* W.H. Freeman and Company.

Two polynucleotides or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by

corresponding nucleic acids which encode the given peptide. Provided with a peptide sequence of the invention, one of skill will recognize a variety of equivalent nucleic acids which encode the peptide. This is because the genetic code requires that each amino acid residue in a peptide is specified by at least one triplet of nucleotides in a nucleic acid which encodes the peptide. Due to the degeneracy of the genetic code, many amino acids are equivalently coded by more than one triplet of nucleotides. For instance, the triplets CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is to be encoded by a nucleic acid triplet, the nucleic acid has any of the triplets which encode arginine. One of skill is thoroughly familiar with the genetic code and its use. An introduction to the subject is found in, for example, chapter 15 of Watson, *et al.*, *Molecular Biology of the Gene* (Fourth Edition, The Benjamin/Cummings Company, Inc., Menlo Park, California (1987)), and the references cited therein, the teachings of which are incorporated herein by reference for all purposes.

Although any nucleic acid triplet or Acodon@ which encodes an amino acid can be used to specify the position of the amino acid in a peptide, certain codons are preferred. It is desirable to select codons for elevated expression of an encoded peptide, for example, when the peptide is purified for use as an immunogenic reagent. Codons are selected by reference to species codon bias tables, which show which codons are most typically used by the organism in which the peptide is to be expressed. The codons used frequently by an organism are translated by the more abundant t-RNAs in the cells of the organism. Because the t-RNAs are abundant, translation of the nucleic acid into a peptide by the cellular translation machinery is facilitated. Codon bias tables are available for most organisms. For an introduction to codon bias tables, *see*, *e.g.*, Watson, *et al.*, *supra.*

C. Making Conservative Substitutions

In addition, it will be readily apparent to those of ordinary skill in the art that the immunogenic HAV peptides of the present invention and the nucleic acid encoding such immunogenic peptides can be subject to various changes, such as insertions, deletions, and substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use, *i.e.*, to increase biological activity.

One of skill will appreciate that many conservative variations of nucleic acid constructs yield a functionally identical construct. For example, due to the degeneracy of the genetic code, Asilent substitutions@ (*i.e.*, substitutions of a nucleic acid sequence which do not result in an alteration in an encoded peptide) are an implied feature of *every* nucleic acid sequence which encodes an amino acid. In addition, one of skill will recognize many ways of generating alterations in a given nucleic acid construct. Such well-known methods include site-directed mutagenesis, PCR amplification using degenerate

oligonucleotides, exposure of cells containing the nucleic acid to mutagenic agents or radiation, chemical synthesis of a desired oligonucleotide (*e.g.*, in conjunction with ligation and/or cloning to generate large nucleic acids) and other well-known techniques. *See,* Giliman and Smith (1979) *Gene* 8:81-97, Roberts *et al.* (1987) *Nature* 328:731-734 and Sambrook, Ausbel, Berger and Kimmel, *all supra.*

Modifications to nucleic acids are evaluated by routine screening techniques in suitable assays for the desired characteristic. For instance, changes in the immunological character of encoded peptides can be detected by an appropriate immunological assay. Modifications of other properties such as nucleic acid hybridization to a complementary nucleic acid, redox or thermal stability of encoded proteins, hydrophobicity, susceptibility to proteolysis, or the tendency to aggregate are all assayed according to standard techniques.

Similarly, Aconservative amino acid substitutions,@ in one or a few amino acids in an amino acid sequence of a protein are substituted with different amino acids with highly similar properties (*see*, the definitions section, *supra*), are also readily identified as being highly similar to a disclosed construct. By conservative substitutions is meant replacing an amino acid residue with another which is biologically and/or chemically similar, *e.g.*, one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are a feature of the present invention.

D. Immunogenic Conjugates

In another embodiment the present invention provides immunogenic conjugates, the immunogenic conjugates comprising an immunogenic HAV peptide covalently attached to a carrier protein. Suitable carrier proteins include, but are not limited to, the following thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(D-lysine:D-glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine, *etc.*

Where the immunogenic peptide and the carrier protein are relatively short in length (*i.e.*, less than about 50 amino acids), they are often synthesized using standard chemical peptide synthesis techniques. Where both molecules are relatively short, a chimeric molecule is optionally synthesized as a single contiguous polypeptide. Alternatively, the immunogenic peptide and the carrier molecule can be synthesized separately and then fused chemically.

Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino

Replaced by Article 34